SEASONAL CHANGES IN COLD-HARDINESS OF FUCUS VESICULOSUS

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Some marine algae, such as the fucoids, grow attached above mean low water and are, therefore, subjected at ebb tide to rather severe atmospheric conditions in some climates. In winter in the arctic, species of *Fucus* may be subjected to air temperatures down to − 40° C., yet do not seem to be at all injured (Scholander, Flagg, Hock and Irving, 1953; Kanwisher, 1957). The drying resistance of marine algae has been studied frequently in the past and it is apparent that those growing highest in the littoral zone are generally the most desiccation-resistant (Muenscher, 1915; Pringsheim, 1923; Isaac, 1935; Stocker and Holdheide, 1937; Biebl, 1939). The fact that they are desiccation-resistant suggests that they might also be fairly cold-resistant. This idea is borne out by findings of Kylin (1917) with *Fucus* and by those of Biebl (1958) with various marine algae.

In spite of this work, there appear to have been no seasonal studies of cold-hardiness of the common littoral marine algae. A number of such studies have been made with land plants (for example, Parker, 1955, 1959), and these have shown that there are wide fluctuations in cold-hardiness with the season in all woody plants of relatively cold climates. It therefore seemed of general biological interest to determine whether a marine plant such as *Fucus vesiculosus* L. would also go through such fluctuations.

Methods

From June of 1958 to late May of 1959 *Fucus* plants were collected from convenient locations along the Long Island shore, near New Haven, as a preliminary study. Although there appeared to be a definite trend in hardiness from about − 20° C. in August to about − 60° C. in February, results were so irregular that it was suspected that some other environmental factor or factors besides air temperature were involved. It was therefore decided to repeat these experiments another year but to obtain the plants from a single location, both as regards the harbor and the position above low tide. It was found that hardiness varied as much as 15° C. in May between plants brought from about the mean low tide level and those brought from the highest fucoid zone, the higher ones being more resistant. This latter zone was used in the 1959–1960 experiments which are reported in this paper. It was decided that the stone jetty at Hammonasset beach, near Madison, Connecticut, was the best location, since it was accessible and the water relatively clean. An attempt was also made to obtain the plants as near to 2:00 P.M. as possible, in spite of variable tide levels for a particular time of day at different times of the year.

Fucus plants about eight inches long were plucked from the rock, put in stoppered jars of fresh sea water, and taken to the laboratory within 30 minutes. Fronds three inches long, including reproductive tips as well as vegetative ones, were cut from these plants, blotted on filter paper, and put in 500-ml. Dewar flasks in a damp condition. These were stoppered with a cork and cooled by an ethane compression system in a low temperature apparatus having a two-foot square compartment (Parker, 1959). The ethane, in turn, was cooled by a Freon-22 compressor system (Cincinnati Sub-Zero Products, Ohio). After being cooled at a rate of

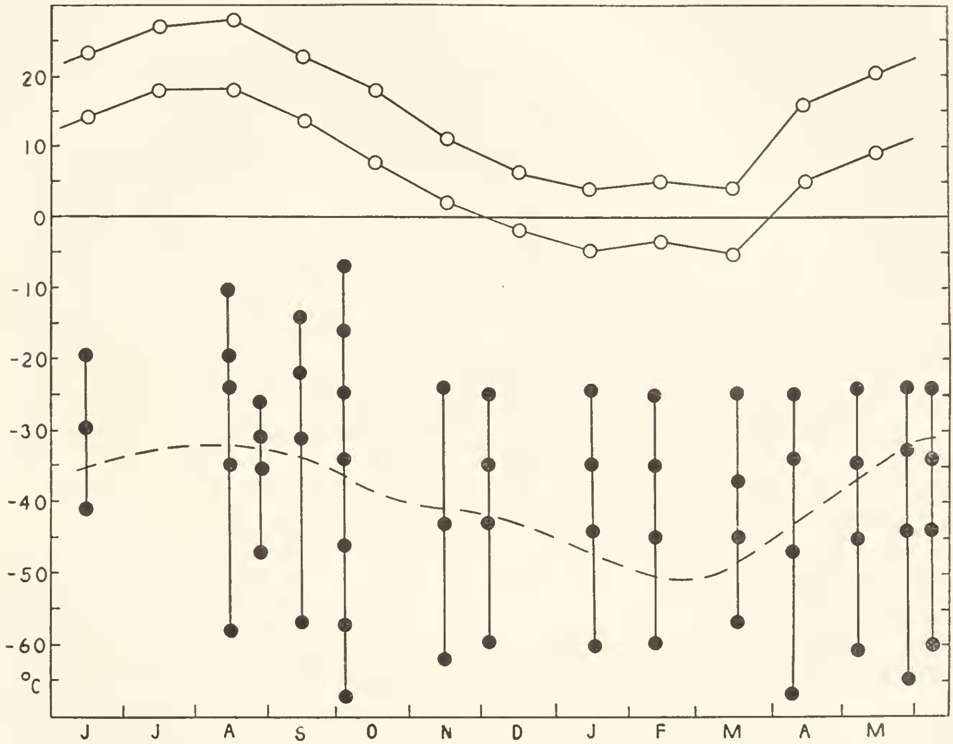

FIGURE 1. Upper curves represent maximum and minimum temperatures at the New Haven municipal airport, averaged for each month for the 1959–1960 season. Lower curve (dashed) represents the relative hardiness of *Fucus,* drawn rather freely as the approximate point at which 50% of the frond was alive (reducing tetrazolium chloride). Data are given in Table I. Blacked-in circles represent temperatures to which a group of plants were cooled on a particular day.

4° C. per hour to one of the temperatures indicated by the blacked-in circles in the results (Fig. 1), flasks were removed to an ordinary refrigerator for three hours and then to room air to obtain a warming rate of about 8° C. per hour.

Determination of viability by ordinary means, for example by leaf color, is very difficult in these marine algae and may account for the lack of research in this field. But by means of the tetrazolium test, very clear and reliable results could be obtained. The fact that reduction of this compound by dehydrogenases to its red formazan derivative is a good indication of cellular viability has been previously discussed (Parker, 1953a, 1953b). One-inch-long cuttings from the tops of the cold-treated frond material were placed in 8-ml. test tubes and 6 ml. of a solution added, consisting of a 1 : 1 mixture of sea water and 0.6% 2,3,5-triphenyl tetrazolium chloride in tap water. Tubes were stoppered and placed in the dark for 4 to 18 hours at 23° C. Although some results can be observed in 4 hours, it was found best to wait 18 hours. After about 24 hours, bacterial action commonly interferes with results and clouds up the water with red precipitate. Reliability of the tetra-

TABLE I

Data shown in the lower part of Figure 1, together with effects of tetrazolium (TTC) test

June 15	Temp.* TTC test**	−19 100	−29 100	−42 0				
Aug. 15	Temp. TTC test	−10 100	−19 100	−24 100	−36 40	−58 0		
Aug. 29	Temp. TTC test	−26 100	−31 50	−36 40	−48 5			
Sept. 10	Temp. TTC test	−14 100	−23 100	−32 40	−57 0			
Oct. 5	Temp. TTC test	−8 100	−17 100	−25 100	−34 100	−46 5	−57 0	−67 0
Nov. 15	Temp. TTC test	−24 100	−43 5	−62 0				
Dec. 4	Temp. TTC test	−25 100	−35 100	−42 10	−59 5			
Jan. 15	Temp. TTC test	−24 100	−35 100	−43 80	−59 10			
Feb. 12	Temp. TTC test	−25 100	−35 100	−44 80	−59 15			
Mar. 18	Temp. TTC test	−25 100	−37 100	−44 60	−57 50			
Apr. 10	Temp. TTC test	−25 95	−35 100	−47 10	−67 5			
May 9	Temp. TTC test	−24 100	−35 70	−45 5	−61 3			
May 31	Temp. TTC test	−24 100	−32 5	−44 0	−65 0			
June 6	Temp. TTC test	−24 100	−34 5	−44 0	−60 0			

* Temperature in degrees centigrade to which fronds were cooled.
** Frond surface in % showing positive tetrazolium test.

zolium test was also supported by the fact that *Fucus* failing to reduce the compound emitted a rotten odor in 48 hours while those reducing it did not.

Results

Cold-hardiness was at a minimum during the summer at about − 30° C. (Fig. 1). Hardiness increased by October and by late winter reached about − 50° C. Since there were frequently borderline cases of damage, it was necessary to express results in terms of percentage tissue area reducing tetrazolium chloride (Table I). The dashed line in Figure 1 is drawn at approximately the point where there was 50% of the tissue not injured (reducing tetrazolium chloride). But even this system was somewhat complicated by the fact that in late summer, growing tips were more sensitive to cold than the rest of the plant, whereas in winter and early spring, tips appeared to be more hardy than the rest of the plant.

In spring, dehardening began to appear by mid-April when woody land plants were showing rapid dehardening. This was apparently related to the higher air temperatures of spring, beginning near the end of March. Surface water temperatures measured at the western end of Long Island Sound (Anon., 1947) and plotted as monthly means for a year showed little deviation from the air temperature data shown in Figure 1. It is therefore impossible to say whether air or water temperature is better related to changes in hardiness. There is, of course, no proof that seasonal changes in hardiness are not controlled endogenously or by some other environmental factor such as day-length.

Summary

A seasonal study of the changes in cold-hardiness of *Fucus vesiculosus* L. was made over a two-year period. Plants in summer could withstand about − 30° C. (the lowest temperature at which 50% of the frond was still alive after treatment), whereas in January and February, plants could withstand − 45° C. to nearly − 60° C. Changes in hardiness with the season appeared to be related to air temperature variations, but also to mean monthly surface water temperatures plotted for a year. In May, *Fucus* taken from the low tide level was 15° C. more sensitive to the cold treatment than those from the highest level of the fucoid zone. Growing tips were the hardiest part of the plants in winter and early spring, but the least hardy in late summer.

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